

Applicant : Leif Andersson et al.
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Attorney's Docket No.: 11145-007001

In the specification:

Please insert the following paragraph at page 1, line 1 of the specification:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application 60/195,665, filed April 7, 2000.

Please amend the paragraph beginning at page 2, line 1 as follows:

Several isoforms of the three different AMPK subunits are present in mammals. An *RN* allele in Hampshire pigs is associated with a non-conservative mutation in a gene encoding a muscle-specific isoform of the AMPK γ chain. In humans, *PRKAA1* on human chromosome (HSA) 5p12 and *PRKAA2* on HSA1p31 respectively encode isoforms $\alpha 1$ and $\alpha 2$ of the α subunit, *PRKAB1* on HSA12q241, and *PRKAB2* (not yet mapped) respectively encode isoforms $\beta 1$ and $\beta 2$ of the β subunit, and *PRKAG1* on HSA12q13.1 and *PRKAG2* on HSA7q35-q36 respectively encode isoforms $\gamma 1$ and $\gamma 2$ of the γ subunit (OMIM database, <http://www.ncbi.nlm.nih.gov/omim/>, worldwide web at [ncbi.nlm.nih.gov/omim](http://www.ncbi.nlm.nih.gov/omim/), July 1999). A third isoform ($\gamma 3$) of the γ subunit of AMPK also is present. Milan et al., *Science*, 2000, in press; and Cheung et al., *Biochem. J.*, 2000, 346:659-669. Analysis of the sequences of these γ subunits shows that they include four cystathione β synthase (CBS) domains whose function is unknown.

Please amend the paragraph beginning at page 3, line 30 as follows:

FIG 1 is an 821 bp DNA sequence of *PRKAG3* (SEQ ID NO:1) from the 5' untranscribed and untranslated region (UTR) through intron 2, including exon 1 and 2.

Please amend the paragraph beginning at page 4, line 1 as follows:

FIG 2 is a 989 bp DNA sequence of *PRKAG3* (SEQ ID NO:2) from intron 2 through intron 4, including exons 3 and 4.

Please amend the paragraph beginning at page 4, line 3 as follows:

FIG 3 is a 1722 bp DNA sequence of *PRKAG3* (SEQ ID NO:3) from intron 4 through intron 10, including exons 5-10.

Please amend the paragraph beginning at page 4, line 5 as follows:

FIG 4 is a 1014 bp DNA sequence of *PRKAG3* (SEQ ID NO:4) from intron 10 through the 3'-UTR, including exons 11-13.

Please amend the paragraph beginning at page 4, line 7 as follows:

FIG 5 is the complete coding sequence of *PRKAG3* (nucleotides 20 – 1489) (SEQ ID NO:5) and the amino acid sequence of the *PRKAG3* polypeptide (SEQ ID NO:6).

Please replace Table 1 on page 13 with the following Table.

TABLE 1
Primer Sequences

Primer name	Orientation	Sequence 5'-3'	SEQ ID NO	Location
hRNF12	Forward	AGG CTC TTG GAA TAG GGG CTC AGG	7	5'untranscribed
nRNR13	Reverse	AGG GAA TTG GGG TCC CAG AAA AGT G	8	intron 2
hRNF1	Forward	GAATTGATTTTGATGCATTACTCC	9	intron 2
hRNR1	Reverse	AGTGCGGCTGCAGCACCGT	10	intron 4
hRNF2.2	Forward	AGG CAG ATG GGA GGT GCG CAC TGA G	11	Intron 4
hRNR2.2	Reverse	ACA GGG ATG GCA TGA GAA ACC CTG C	12	Intron 10
hRNF4.2	Forward	TTC TGG TAG TGG CAC CCT GAT GCA A	13	Intron 10
hRNR3.2	Reverse	GAC CTG TGA GTC CTT ACA CTT GCA G	14	3'UTR

Please replace Table 4 on page 16 with the following Table:

TABLE 4
Single nucleotide polymorphisms in the human *PRKAG3* gene

Location	Nucleotide position	Nucleotide change	Predicted amino acid change ^a
exon 3	230 ^a	C \leftrightarrow G	P71A
exon 4	559 ^a	C \leftrightarrow T	No
intron 6	642 ^b	C \leftrightarrow G	--
exon 10	1037 ^a	C \leftrightarrow T	R340W

^a Position based on the human cDNA sequence in Figure 5.

^b Nucleotide position based on the sequence in Figure 3.

Please amend the paragraph beginning on page 17, line 3 as follows:

A variety of available molecular genetic techniques for SNP detection can be used to screen the SNPs in Table 4, as described above. PCR primers hRNF9 (5' GCT GGA TCC CG ATC TCC ACC TG, forward, intron9, SEQ ID NO:15) and hRNR10(5'CGT TGA CCA CAG GCA GTG CAG AC, reverse, exon10, SEQ ID NO:16) were designed from the FIG 3 sequence and used for PCR amplification of a 200 bp fragment containing the SNP in exon 10. The PCR reactions were performed in 10 μ l reactions including 0.35 U *AmpliTaq* DNA polymerase (Perkin Elmer, Branchburg, NJ, USA), 1x PCR buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 2.5 pmol of each primer, 5% DMSO, and 10 ng genomic DNA. Thermocycling was carried out using a PTC 100 instrument (MJ Research, Watertown, MA, USA). The thermocycling included 40 cycles with annealing at 61°C for 30 s and extension at 72°C for 30 s. The denaturation step was at 95°C for 2 min in the first cycles, and at 94°C for 1 min in the remaining cycles. Four μ l of each PCR product were digested in 10 μ l with 2.4 U MspI (New England Biolabs, Frankfurt am Main, Germany) containing the buffer recommended by the manufacturer. The digestions were analyzed by 6% Nusieve/Seakem 3:1 agarose (FMC Bioproducts, Rockland, ME, USA) gel electrophoresis and visualization of the DNA fragments by ethidium bromide staining and UV illumination. Digestion with Msp I generated allelic fragments of 169 bp (allele 1), 114 and 55 bp (allele 2) as well as the monomorphic fragment 31 bp. Homozygous 2/2 genotypes and heterozygous 1/2 genotypes were observed.